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Effect of administration of ajwa date extract on renal histopathological changes in meloxicam induced in rats

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Abstract

This study aimed to examine the nephroprotector effect of ajwa date extract on the kidneys of white rats induced by meloxicam. 25 male (Rattus norvegicus) rats weighing 200-300 g were divided into 5 groups (n = 5), namely group 1 healthy control without meloxicam and ajwa date extract, group 2 negative control with meloxicam 30mg/kgBB without ajwa dates, group 4 with ajwa date extract 150mg/kgBW and meloxicam 30mg/kgBW, and group 5 with ajwa date extract 300mg/kgBW and meloxicam 30mg/kgBW. The treatment was given for 14 days, on the 15th day a necropsy was performed to take kidney organs for microscopic evaluation or histipathological examination. Meloxicam at a dose of 30mg/kgBW caused damage to the kidneys with observations of glomerular hemorrhage, tubular necrosis, hydropic degeneration and narrowing of the tubular lumen as well as narrowing of the capsular space with an average degree of damage reaching 75% while ajwa date extract doses of 150 and 300mg/kgBW has a nephroprotector effect against meloxicam induction. Ajwa date extract doses of 150 and 300mg/kgBW had a nephroprotector effect on meloxicam induction.

Keywords: Ajwa date extract; meloxicam; kidney; nephroprotector

Introduction

Meloxicam is a non-steroidal anti-inflammatory drug that works by inhibiting COX-2. COX-2 as an enzyme in charge of converting prostaglandin H2 into prostaglandin E2 which plays a role in the incidence of inflammation, pain and fever. Prostaglandins in the kidneys function to maintain salt and water homeostasis and to maintain blood flow to the kidneys.

The clinical side effects of using oxicam class of drugs are decreased sodium excretion, decreased potassium excretion and decreased renal perfusion. Decreased sodium excretion can lead to peripheral oedema, hypertension, and usually chronic heart failure. Hyperkalemia can occur, causing cardiac arrhythmias. Renal function becomes decreased, resulting in acute kidney failure. Renal function failure in the form of interstitial nephritis or papillary necrosis (Brater 1999).

Giving meloxicam above the normal recommendation, there will be an acute or chronic meloxicam overdose which can cause damage to the liver, kidneys and also gastrointestinal ulcers (Joseph 2011). Research conducted by Adleend (2015) showed that giving meloxicam at a dose of 30 mg/kg BW for 4 consecutive days in male rats showed kidney damage. The histopathological picture of the kidney found the presence of cell death (pyknosis and necrosis), the presence of hemorrhage and vacuolization in the glomerulus and renal tubules. Dates or Phoenix dactylifera L are a good source of phytochemicals, including carotenoids, phenolics, and flavonoids. Dates can not only provide antioxidant, antimutagenic, and immunomodulatory benefits for health but also have diverse medicinal values, including antihyperlipidemic, anticancer, gastroprotective, hepatoprotective, and nephroprotective properties (Talhouk et al. 2007). Various studies have shown that dates or Phoenix dactylifera L contains antioxidants (El Arem 2014), has a gastroprotective effect through the administration of ethanol to induce gastric ulcers in wistar rats, hepatoprotective where dates are able to protect the liver from hepatotoxicity due to CCl4 induction in mice (Abdelaziz et al. 2014), nephroprotective where dates are able to protect kidney from gentamicin-induced nephrotoxicity in mice (Al-Qarawi et al. 2008), and has anti-cancer activity (Khan et al. 2017). The results of the study that explained the efficacy of dates were research conducted by Al-Erem et al (2014), the results of the research conducted explained that date palm extract (*Phoenix dactylifera*) had effectiveness as a nephroprotector against dichloroacetic acid-induced nephrotoxicity in adult rats. The administration of dichloroacetic acid in rats caused an increase in malondialdehyde levels in the kidneys, but the administration of date fruit extract decreased malondialdehyde levels, decreased urea and creatinine levels through the capacity of dates as antioxidants. In addition, Jamila (2015) in his research said that the date palm extract had a nephroprotective effect on exposure to Rhodamine B by finding an improvement in the glomerulus and kidney tubules. This study also concluded that the nephroprotective ability of dates against kidney damage is due to the high antioxidant effect of dates (Jamila 2015). Based on the description above, the researchers felt the need to conduct research on the effect of giving ajwa date fruit extract on kidney damage in white rats (*Rattus norvegicus*) induced by meloxicam. Most studies mention the nephroprotective effect of dates on kidney damage induced by compounds or free radical causative agents. In addition, research that specifically mentions the nephroprotective effect of Ajwa variety dates is still very limited.

Meterials and Methods

Statement of Ethics

The research process was carried out by following the Guide for the Care and Use of Laboratory Animals, Edition 8, by the Institute for Laboratory Animal Research 2011 and approved by the ethical commission of the Faculty of Medicine, Hasanuddin University (Number: 190/UN4.6.4.5.31/PP36/2022). Selection, acclimatization and rearing of experimental animals 25 male Rattus norvegicus white rats weighing 200-300g were divided into five groups (n = 5). Subjects were treated in a laboratory controlled for temperature, air pressure, humidity, and dark and light cycles every 12 hours and were given standard feed and drinking ad libitum.

Tools and Materials

Olympus X5201 microscope, ajwa date fruit extract, meloxicam, neutral buffered formalin 10%, alcohol 90%, alcohol 80%, alcohol 70%, acid alcohol, xylol, hematoxylin, and eosin. Giving ajwa date extract and meloxicam Group 1 was not given ajwa date extract or meloxicam, group 2 was given meloxicam on days 1-14. Groups 3, 4, and 5 were given ajwa date extract at doses of 75, 150, and 300 mg/kgBW and meloxicam 30mg/kgBW was given on days 1-14 then on day 15 the kidneys were removed.

Tissue Sampling and Examination

Each experimental animal from each group was euthanized under anesthesia using a combination of ketamine-xylasin followed by abdominal surgery to remove the kidney. The fixed specimens were then dehydrated in graded alcohol, clarified in graded xylol, embedded in paraffin, cut into ±5 m thin strips, and stained with hematoxylin and eosin (H&E). Observation of changes in renal histology was carried out under a microscope.

Results and Discussion

Kidney histopathological preparations were made on the 15th day by taking samples through necropsy. The observations obtained are as follows (table 1):

Table 1. Average of degrees of kidney damage in each group

Group	Average degree of kidney damage
K1	0
K2	2.8
K3	2.2
K4	1.4
K5	1.2

Note: 0=no change; 1=slight damage or cell damage reaching 25%; 2=moderate damage or cell damage reaches 50%; 3 = severe damage or cell damage reaches 75%.

Comparison of the degree of kidney damage in each group can be seen in the graph in Figure 1.

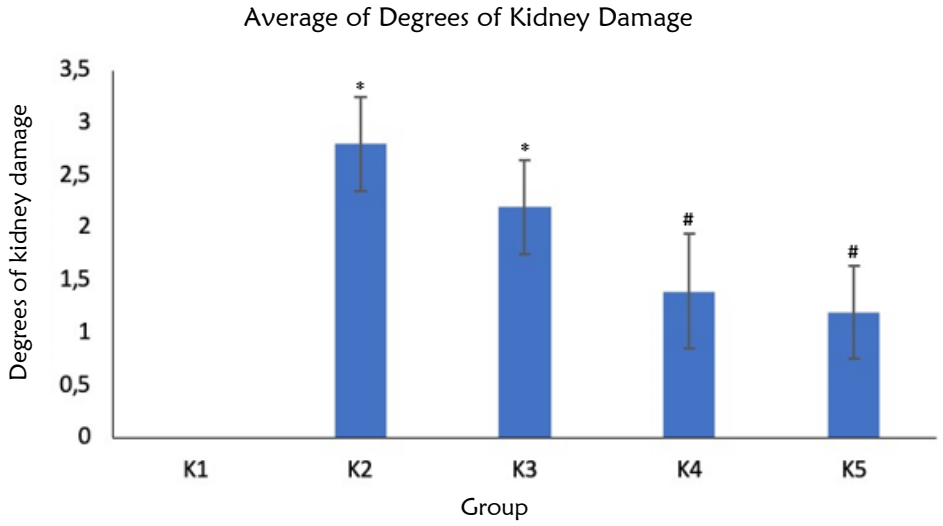

Figure 1. Diagram of the average kidney damage in each treatment group. The symbol * indicates $p<0.05$ (significant) against negative controls, symbol # indicates $p<0.05$ (significant) against

negative and positive controls.

In figure 1.2. It was seen that the renal histology of the K1 group (negative control) which was not induced by meloxicam nor the ajwa date fruit extract was not damaged (degree of damage 0 or normal).

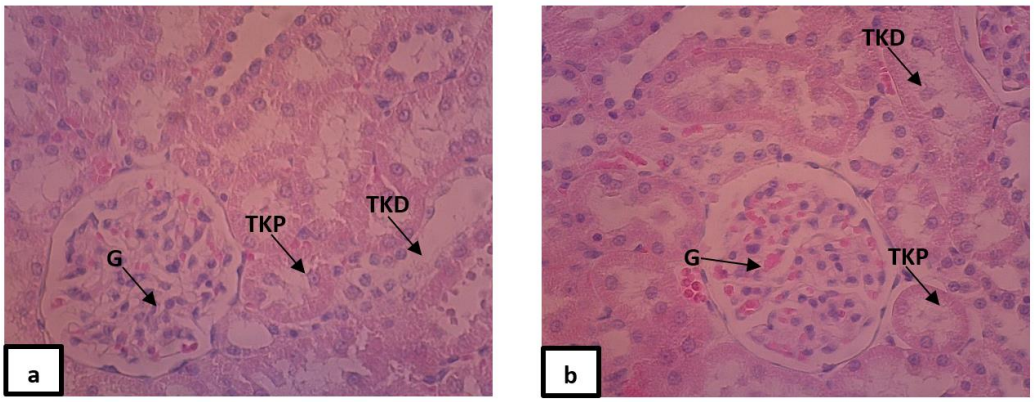

Figure 2. Histology of the kidney of rats in the K1 negative control group (HE, 40X magnification). G: Glomerulus, TKD : Proximal convoluted tubule, TKD : Distal convoluted tubule). In pictures a and b, the glomerulus is normal (the nucleus is clearly visible and the glomerulus is round). Normal proximal convoluted tubule (cells are not swollen, cell nucleus is round, tubular cell lumen is clear)

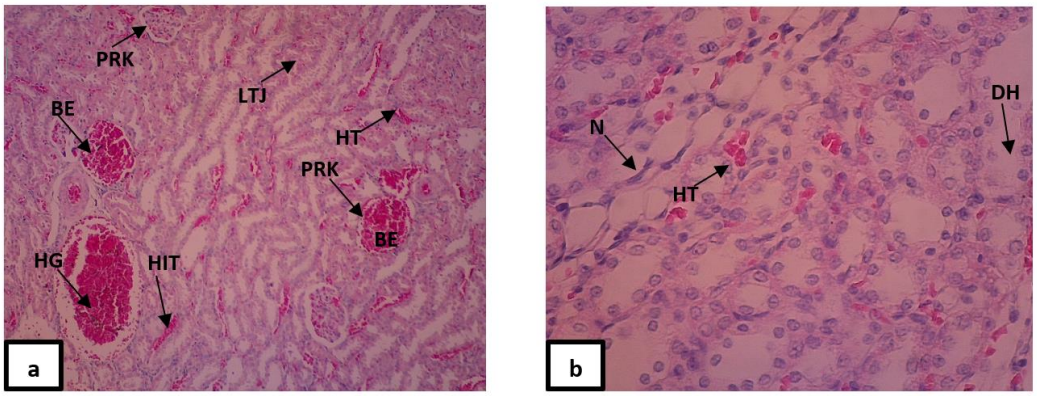

Figure 3. Histological picture of the kidneys of the K2 positive control group rats (HE, 10X and 40X magnification). Figure (a) shows glomerular hemorrhage (HG), capsular space narrowing (PRK), erythrocyte grains in the glomerulus (BE), tubular hemorrhage (HT), intertubular hemorrhage (HIT) and unclear tubular lumen (LTJ) with the degree of damage. 3 or severe: cell damage reaches 75%. Figure (b) shows cell necrosis (N), hydropic degeneration (DH) and tubular hemorrhage (HT) with a degree of damage 3 or severe: cell damage reaches 75%.

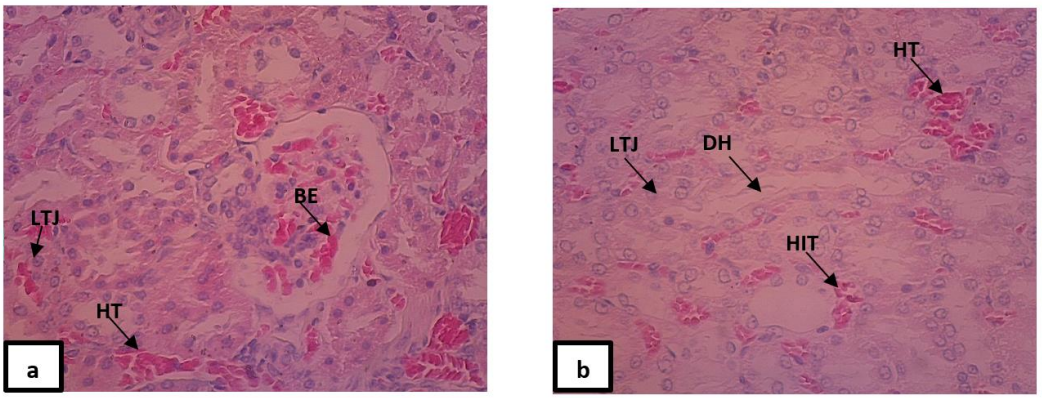

Figure 4. Histology of the kidney of K3 group rats (HE, 40X magnification). Figure (a) shows tubular hemorrhage (HT), intertubular hemorrhage (HIT), erythrocyte grains in the glomerulus (BE), and tubular lumen is unclear (LTJ) with a degree of damage 2 or moderate: cell damage reaches 50%. Figure (b) shows hydropic

degeneration (DH), unclear tubular lumen (LTJ) and tubular hemorrhage (HT) with a degree of damage 2 or mderate: cell damage reaches 50%.

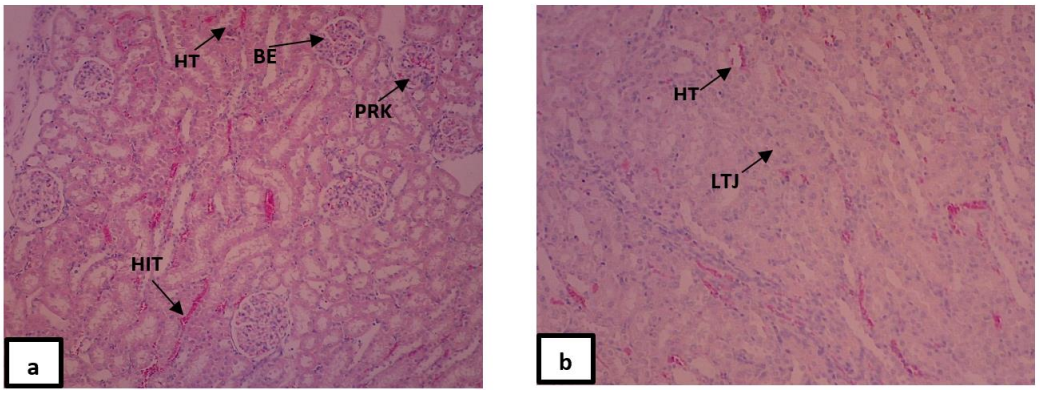

Figure 5. Histology of the kidney of K4 group rats (HE, 10X magnification). Figure (a) shows tubular hemorrhage (HT), intertubular hemorrhage (HIT), erythrocyte grains in the glomerulus (BE), and capsular space narrowing (PRK) with a degree of damage 1 or mild: cell damage reaches 25%. In the picture (b) it is seen that the tubular lumen is not clear (LTJ) and tubular hemorrhage (HT) with a degree of damage 1 or mild: cell damage reaches 25%.

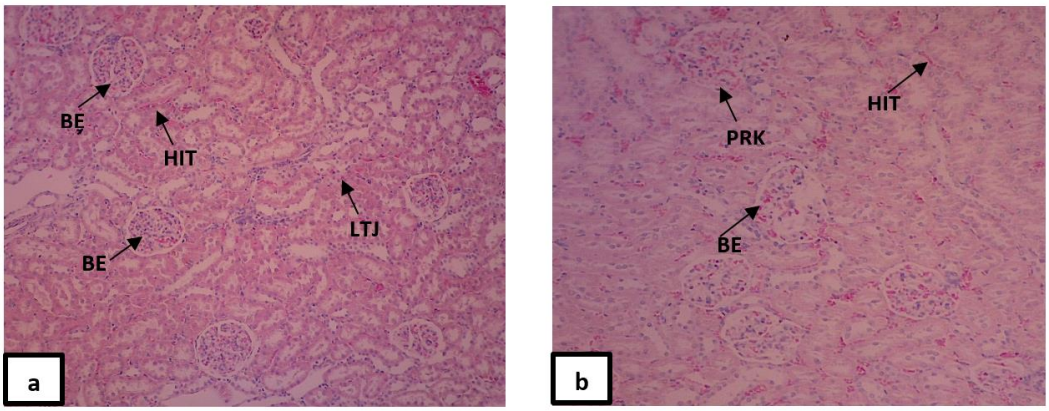

Figure 6. Histology of the kidney of K5 group rats (HE, 10X magnification). In the picture (a) intertubular hemorrhage (HIT), erythrocyte grains in the glomerulus (BE), and unclear tubular lumen (LTJ) with a degree of damage 1 or mild: cell damage reaches 25%. Figure (b) shows erythrocyte grains in the glomerulus (BE), capsular space narrowing (PRK) and intertubular hemorrhage (HIT) with a degree of 1 or mild damage: cell damage reaches 25%.

The results of histopathological examination of the kidneys showed that the healthy control group or K1 showed a score of 0 or no damage occurred while the negative control or K2 (meloxicam 30mg/kgBW) showed the degree of severe damage. Histopathological analysis was performed using a statistical test using Kolmogorov Smirnov, then continued with the Mann Whitney U test. From the statistical test it was found that the positive control group had a significant difference with the negative control group ($p < 0.001$). In addition, it was found that the K4 group (Ajwa dates 150 mg/kg BW) and the K5 group (Ajwa dates 300mg/kgBW) had a significant difference to the positive control ($p < 0.05$) while the K3 group (Ajwa dates 75mg/kgBW) had a significant difference. not significantly different from the positive control ($p < 0.05$).

Histopathological examination of the kidneys found that the healthy control group K1 showed no abnormalities in the kidney organs which microscopically showed clear and rounded glomeruli without swelling, capsular space narrowing and hemorrhage and proximal and distal convoluted tubules had clear lumens and no changes. In this group, the damage score was 0. In the negative control group, K2 (meloxicam 30mg/kgBW) showed massive damage with damage scores of 3 and 2. In this group, it was found that glomerular

hemorrhages, capsular space narrowing, erythrocyte grains in the glomerulus, tubular hemorrhages were seen. , intertubular hemorrhage, tubular lumen is not clear, visible cell necrosis, degeneration with degrees of damage 3 and 2: cell damage reaches 50-75%. K3 group (Ajwa date extract 75mg/kgBW and meloxicam) the degree of damage was scored 2 or moderate with cell damage reaching 50%. Group K4 (ajwa date extract 150mg/kgBW and meloxicam) and group K5 (ajwa date extract 300mg/kgBW and meloxicam) showed a degree of damage 1 or mild with cell damage reaching 25%. The lowest mean degree of kidney damage was group K1, then K5, K4, K3 and the highest was K2.

Histopathological analysis was carried out with statistical tests using Kolmogorov Smirnov, then continued with the Mann Whitney U test. From statistical tests it was found that the healthy control group had a significant difference with the negative control ($p < 0.001$), meaning that the administration of meloxicam caused damage to kidney tissue. In addition, it was found that the K4 group (ajwa dates 150 mg/kg BW) and the K5 group (Ajwa dates 300mg/kgBW) had a significant difference to the negative control ($p < 0.05$) while the K3 group (Ajwa dates 75mg/kgBW) not significantly different from the negative control ($p < 0.05$), meaning that the administration of 150 mg and 300 mg of Ajwa dates had a protective effect against kidney damage caused by meloxicam.

The kidneys to carry out this excretory function have a heavy task, because almost 25% of all blood flow flows to the two kidneys (Guyton and Hall 2006). Toxic substances will easily cause damage to kidney tissue in the form of changes in renal structure and function. Toxicants that enter the kidney can cause various abnormalities in the structure and function of the nephron. Damage to the nephrons can occur in the tubules, renal corpuscles, and blood capillaries in the kidneys (Husein and Trihono 1996). Disorders of the corpuscle can damage the glomerulus and Bowman's capsule, so that it will interfere with the smooth flow of blood in the glomerular capillaries. Damage to the tubules can occur in epithelial cells, including degeneration and atrophy so that the lumen widens. Further damage can result in nephron death (Ressang 1984). Nephron death occurs as a result of cell degeneration. Cell degeneration is the deterioration of cells that causes changes in form and function (Himawan 1996).

Based on research, meloxicam is toxic to kidney cells by interfering with mitochondrial function by scattering mitochondrial membranes and inhibiting ATP biosynthesis due to its ability to inhibit aspartate thereby limiting the bioavailability of the two main substrates glutamate and malate. Due to decreased mitochondrial function, it is not surprising that meloxicam can result in nephrotoxic cell death (Lin Eng 2008). Cell death can be caused by various factors, one of which is hypoxia due to disruption of the circulation system by toxic substances that enter. In addition to hypoxia, cell death can also be caused by ischemia. The tubular damage caused by ischemia varies greatly depending on the extent and duration of the decrease in renal blood flow. Necrosis begins with changes in the morphology of the cell nucleus, namely pyknosis. The next stage is the nucleus ruptures (kariorexis) and the nucleus disappears (karyolysis). Pyknosis can occur due to damage in cells, including membrane damage followed by damage to mitochondria and the Golgi apparatus so that t cells (Price 2006).

Ajwa dates (Phoenix dactylifera) have extraordinary benefits for the body such as preventing hypertension, coronary heart disease, obesity, hyperlipidemia and diabetes (Ragab et al. 2013). These ingredients influence each other in pharmacological effects such as anticancer, antioxidant, antiulcerative, anti-inflammatory, antiproliferative, antimutagenic, antibacterial and antifungal. The results of research conducted by Al-Erem et al (2014) explained that date palm extract (Phoenix dactylifera) has effectiveness as a nephroprotector against dichloroacetic acid-induced nephrotoxicity in adult rats (Sani et al.

2015). The administration of dichloroacetic acid in rats caused an increase in malondialdehyde levels in the kidneys, but the administration of date fruit extract decreased malondialdehyde levels, decreased urea and creatinine levels through the capacity of dates as antioxidants. In addition, Jamila (2015) in his research said that the date palm extract had a nephroprotective effect on exposure to Rhodamine B by finding an improvement in the glomerulus and kidney tubules. This study also concluded that the nephroprotective ability of dates against kidney damage is due to the high antioxidant effect of dates. Ajwa dates have been shown to maintain the highest antioxidant activity among other types of dates, suppress lipid peroxidation, prevent damage (Sahyon and Al Harbi 2020). As is known, one of the processes of kidney damage caused by excessive administration of meloxicam is the production of free radicals which are highly nephrotoxic. Ajwa dates play a role in suppressing the production of free radicals and oxidative stress through their role as antioxidants in the kidneys, thereby helping to prevent the development of kidney damage due to meloxicam. The results of this study are expected to be useful as reference material and future references to further develop research related to the protective effect of ajwa dates on kidney damage due to meloxicam induction. The results of this study can also be taken into consideration to develop ajwa date extract as a substance to prevent kidney damage, especially for the elderly who have decreased kidney function.

Conclusion

Giving meloxicam at a dose of 30mg/kgBW in rats caused kidney damage. Administration of ajwa date extract at doses of 150 mg/kgBW and 300 mg/kgBW could provide a significant protective effect against meloxicam-induced kidney damage.

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